THE UNIVERSITY OF ALBERTA

LIVER FUNCTION STUDIES USING I-125 ROSE BENGAL Tc-99m SULFUR COLLOID AND I-125 RADIOIODINATED MONO-, DI-, AND TRI-IODOHIPPURIC ACIDS IN NORMAL AND IN UREMIC ANIMAL MODELS

BY

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Abstract

A state of chronic renal failure was induced in rats by long term maintenance of animals which had undergone left sub-total nephrectomy followed by contralateral nephrectomy ten days later. During the three month period following surgery, altered hepatic weight, altered renal mass, decreased serum calcium levels, and many other qualitative changes were characteristically observed in this model. The blood clearance of I-125 rose bengal following intravenous injection, was determined to proceed at a significantly slower rate in uremic rats than in normal controls ($P < 0.025$); total biliary elimination of I-125 rose bengal was also reduced ($P < 0.005$). Significant linear correlation was found between serum calcium levels and I-125 rose bengal clearance rate constants during the one hour elimination.

After intravenous injection of Tc-99m sulfur colloid, a significant increase was recorded in the hepatic uptake rate constant in the rats with chronic renal failure when compared to normal controls. Similarly, a significant increase in Tc-99m sulfur colloid blood clearance was observed in uremic rats.

The total amount of hepatic elimination of the radioactive I-125 labeled 4-iodohippurate, 3,5-diiodohippurate and 3,4,5-triiodohippurate, after

intravenous injection was observed to be increased as the molecular weight of the substrate increased. This effect was more pronounced in subtotally nephrectomized chronically uremic rats than in normal rats. Renal elimination of the I-125 iodohippurate analogues became quantitatively less important as the molecular weight increased, an effect more pronounced in the uremic rats than in normal rats.

Possible implications of these findings in chronic renal failure are discussed.

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I. Introduction

Chronic renal failure is an insidious, progressive disease in which the functions of the kidney are decreased to the point that the kidney can no longer adjust appropriately to ordinary demands of the body. The occurance of the accumulation of waste products and the abnormalities of electrolyte in the body are manifest in a condition called uremia, simply "a generalized symptom with complex syndrome due to a dynamic imbalance between the organism's current metabolism and its appropriate renal function" as defined by Bright back in 1827. When Harrison was performing his classic investigation of uremia in the 1930s, he compared the uremic man with an aging bacterial culture in that both are ultimately destroyed by the poisonous products of their own metabolism.

Statistical reports revealed recently indicated that the incidence of chronic renal failure is 10 per 100,000 and prevalence is 14 - 20 per 100,000. It occurs about equally in men and women. Chronic renal failure may result from any of a large number of disorders which severly damage both kidneys. Pathologic picture and treatment approach varies with the cause and severity of damage to the kidney. Current treatment includes appropriate drug therapy, diet and fluid balance, electrolyte replacement,transfusions, extracorporeal dialysis and kidney transplants. Thousands and thousands of dollars have to be spent per person for a continuous treatment and special medical care. A recent

survey indicates a 1-year survival on dialysis of 87% and a 2-year survival of 73%. Recipient survival with the first kidney transplant still functional at 1 year is 74% when the donor is a parent or sibling, and is about 10% when the transplant is a cadaver kidney. Because the half-life of many drugs is prolonged and a normal dose might become a toxic dose in patients with renal failure, drug therapy is complex and difficult. Close monitoring of the effects of drugs is necessary and the dosage of drugs often must be reduced and guided by blood levels.

Extensive study and research have been taken in the understanding of chronic renal failure and its possible treatment. However, today, little information has been forthcoming with respect to *in vivo* functional integrity of the liver especially during chronic renal failure, nor the role of the liver in detoxication and elimination of xenobiotics which are normally excreted through healthy kidneys, in the above condition. As the liver is one of the major organs in the body handling many dynamic functions including metabolism and excretion, it is therefore logical that we need also a good understanding of liver function during chronic renal failure in order to compliment our present knowledge in the care and treatment of uremia and chronic renal failure as a whole. With the advancement of radioactive tracers and computer analysis of liver and kidney function tests, high accuracy, ease, and minimal

stress or influence to the test organ can be obtained. It is the purpose of this research to employ different radioactive tracers in the study of different aspects of liver function during experimentally induced chronic renal failure. This investigation included:-

a. Sub-totally nephrectomized Wistar rats as animal model;

b. I-125 rose bengal blood clearance and bililary elimination investigations for the assessment of hepatic polygonal cells function, and

c. Technetium-99m sulfur colloid study of hepatic Kupffer cell function.

Knowing the advantages of using radio-iodine labelled hippuric acid as a tracer to estimate the kidney function and the possibility of adding more than one iodine atom to the hippuric acid molecule in order to increase the molecular weight and size, therefore, it was of interest to study also :-

a. whether the animals with renal failure handled the radioactive iodine labelled hippuric acid differently than the normal animals as kidney function parameter, and

b. whether the increase of molecular weight and size in di-iodo and tri-iodo hippuric acid do affect the route of excretion in both normal and renal failure animals to the same extent or completely different. The result obtained might be of significance for future recommendation in the therapeutic management of chronic renal failure.

II. Survey of Literature

1. Chronic Renal Failure

A. Kidney

The kidneys are essential organs in the body for the maintenance of an optimum internal environment so that other organs may function properly. Although the kidneys are frequently referred to as organs of excretion, they are also organs of regulation . In health, the kidney functions to maintain within limits

1. the volume of water in extracellular fluid,
2. the concentration of electrolytes in extracellular fluid,
3. the osmolality of the extracellular fluid, and
4. the concentration of hydrogen ion in the extracellular fluid.

The kidney is also the site for the excretion of the waste of metabolism, particularly those of protein metabolism. Under certain conditions, it regulates the blood pressure through the juxtaglomerular apparatus and the renin-angiotensin system, and also, through the production of erythropoietin by the renal mass, erythropoiesis and maturation of red blood cells can be modified.

B. Chronic Renal Failure and Uremia

When the kidney fails to offer proper function to the

body, the condition is called renal failure. Renal failure may develop suddenly from trauma, prolonged ischemia of the kidney or from the effect of nephrotoxic agents. More commonly, it develops slowly over the course of months from the effects of ischemia secondary to disease of renal arterioles or arteries, from infection of kidney tissue or from other causes. Whatever the cause or causative factors of renal failure in a given patient, the presenting signs and symptoms are due to the effects on all the organ systems of the body resulting from the inability of the kidney to maintain the composition and volume of body fluids as well as its inability to regulate blood pressure and red blood cell maturity.

The syndrome resulting from functional failure of the kidney is known as uremia or azotemia. Uremia is the original term and means, literally, urine in the blood. The term azotemia has a more limited meaning in as much as it refers to the presence of excessive quantities of nitrogenous compound in the blood. The manifestations of uremia are due to the effects of the failure of the kidney to regulate the volume and composition of body fluids in total bodily function.

Metabolic acidosis, intestinal malabsorption of both amino acids and minerals, increased circulating levels of parathyroid hormone (PTH) and uremic toxins such as indoles

and guanidines, alterations in the metabolic fate of carbohydrates as well as the glycogen and glucose utilization (1-5), alterations in lipid metabolism mainfest as hyperlipemia (primarily hypertriglyceridemia) (6,7), altered protein metabolism (8,9) as well as changes in *in vitro* metabolism of xenobiotics (10) have been demonstrated in chronic renal failure. Clinical symptoms include lethargy, anorexia, nausea and vomiting, mental deterioration and confusion, muscle twitching, convulsions, and coma are very common. Anemia (which results from depressed erythropoiesis), bone demineralization, hypocalcemia, high levels of blood urea nitrogen (BUN) and non-protein nitrogen (NPN), as well as elevated level of creatinine are some of the prominent features of chronic renal failure (10,27,125).

C. Experimentally induced chronic renal failure model

The sub-totally nephrectomized, chronically uremic animals have been found to mimic the clinical syndrome of chronic renal failure in many respects, such as accelerated Vitamin D3 turnover, elevated blood urea nitrogen (BUN), bone demineralization, hypocalcemia, anorexia , anemia and delay in clotting time (4,5,6,10,24). Schwiebe (21) and Avioli (22) described at different times the success of contralateral nephrectomy after occlusion of renal vein or ligation of renal artery in the other kidney to produce a

uremic animal model (21,22,47). Kessner and Epstein (23) described a better controlled method involving both a sub-total and total nephrectomy. After carefully freeing the adrenal gland from the left kidney using a midline abdominal approach, 60 to 80% of the functional renal mass was removed. Gelfoam and Surgicel was then sutured in place over the exposed portions of the renal surface. Contralateral nephrectomy was undertaken a week later (23). Further examination and evalution of the uremic models employing the similar approach have been found to mimic uremia likewise (4,5,6,10,24).

Mannan et al. (4,5,6) applied the sub-total nephrectomized rats in the investigation of an alternate parameter (other than blood urea nitrogen) to monitor the early onset of uremia. With the same uremic models, he also studied the deposition of glycogen, structure of liver glycogen, glycogen cycle enzymes, plasma amino acid profile and the oxidation of glucose under the influence of renal failure. Terner et al. (10,24) also applied the similar uremic models in the investigation of changes in drug metabolism and detoxification by hepatic microsomal enzymes as well as the changes of Vitamin D metabolic pattern in sub-total nephrectomized rats in order to gain some insight into the pathophysiology of uremia.

2. Relationship between Liver Function and Chronic Renal Failure

A. Liver

Liver is also one of the dynamic and vital organs with diverse function in the body. It serves as the primary receiving depot, chemical-processing plant and distribution center for almost everything that enters the body through the walls of the alimentary canal. Some of the complex functions include the formation of bile; carbohydrate storage, ketone body formation, and other functions in the control of carbohydrate metabolism; reduction and conjugation of adrenal and gonadal steriod hormones; detoxification of many drugs and toxins; manufacture of plasma protein; inactivation of polypeptide hormones; urea formation and many important functions in the metabolism of fat. Drugs and foreign compounds are metabolized in the liver mainly by oxidations, reductions, hydrolysis and conjugations. The essential effect of such hepatic transformation is to convert lipophilic, or fat-soluble, compounds into hydrophilic, or water-soluble, ones. The hydrophilic compounds are more readily removed from the body by the kidneys and excreted. The liver produces bile, which is a secretion that aids in the digestion of fats when it is released into the small intestine; as well as a vehicle for the excretion of transformed substances and other waste products of metabolism.

B. Inter-relationship of chronic renal failure and liver function

The physiological and pathological inter-relationship between liver and kidney has long been an interesting area for investigation. There are some experimental and clinical observations suggesting that the change of liver function may be associated with renal abnormalities. Altered renal function, with respect to glycogen and glucose utilization (4,5,6), and *in vitro* metabolism of xenobiotics (10), has been demonstrated in the uremic rat. It is suggested that liver damage due to choline deficiency may be associated with severe cortical and medullary necrosis and hemorrhage in the cortex and subcapsular areas of the kidney (11). On the other hand, it is known that patients with liver disease may develop renal failure of unknown cause in the course of their illness (12,13,14,15), and generally signify in a broad term hepatorenal syndrome (16). Renal hemodynamics was suggested to be disturbed specifically as a result of hepatic desease (16). Reversible defect in urine concentrating ability was observed in patients with decompensated cirrhosis (17). Proteinuria, microscopic hematuria, or pyuria has been observed in a significant number of patients with viral hepatitis (18). Impaired water diuresis has been reported during acute hepatitis (19), and renal failure without apparent cause may develop in the

course of Laennecs', cirrhosis (13,14,15).

The presence of renal failure may complicate the management of cirrhotic patients from a therapeutic point of view. The choice and dosage of antibiotics for the prevention and treatment of hepatic incidential infection have to be carefully planned. Some of the work and discussion on the renal capacity for excretion of drugs as well as complications resulting from renal failure in patients with liver disease has been implemented (20).

3. The use of radioactive tracers as diagnostic agents for liver and kidney function tests.

In the liver, about 40% of the liver cell population and 10% of its mass are the phagocytic Kupffer cells which are part of the reticuloendothelial system. Colloidal radionuclide preparations that contain submicron size particles are cleared from the bloodstream by phagocytic Kupffer cells and remain there until they are either metabolized or become "permanently" trapped. The clinical use of the RES "trap" with Technetium-99m-labeled-sulfur colloid to measure liver function is well documented.

Radioactive iodine labeled rose bengal is known to be cleared from the blood by active transport across the membrane of the hepatic polygonal cells which comprise about 60% of liver cells and 90% of liver mass. It is excreted unchanged into the biliary passages and ultimately into the gastrointestinal tract. Normally, no significant re-absorption occurs from the GI tract. However, in the presence of obstruction, radioiodinated rose bengal as well as dissociated radioiodide may be returned to the circulation and excreted by the kidney (37).

Radioactive iodine labeled hippurate which has a very low hepatic uptake is removed and excreted mainly by the healthy kidneys, has long been established as a good agent

for renal fuction evaluation (100,101,102,103). With the comparison of the normal and abnormal renal tracings, evaluation of the renograms produced offers a significant parameter for renal function interpretation. Knowing that the increase of molecular weight of hippurate might affect the normal route of excretion, the examination and evaluation of these changes in both normal and renal failure animal model might be of great significance to the understanding of kidney-liver interrelationship as well as the dynamics of xenobiotic excretion in the condition of renal failure.

Radioactive iodine labeled rose bengal, radioactive technetium-99m labeled sulfur colloid and radioactive iodine labeled hippurate and its higher molecular weight analogues were therefore employed in the excretion investigation in both normal and renal failure animal model.

4. Radio-iodine labeled Rose Bengal

Rose Bengal is a halogenated fluorescein dye and is removed from the blood by the polygonal cells of the liver, secreted into the biliary tract, and finally excreted into the duodenum (32). Stool recovery is nearly complete. With the introduction of radioactive iodine labeled rose bengal, the rates of liver uptake and excretion could be measured externally by gamma ray scintillation counters and appropriate recording devices (33,34,58,59). The hepatogram so recorded can be very useful as a guideline for liver function and hepatic damage as well as for intrahepatic obstruction according to its time course and the shape of the hepatogram (33,34,35,55,56,57).

As rose bengal is found to be loosely bound to plasma proteins, after a single i.v. injection, its rate of removal from the blood is related directly to the function of the polygonal cells and to the liver blood flow. The manner of elimination from the blood is found to be exponential to time. The plasma clearance of rose bengal as a parameter for liver function test is therefore well documented (36,37,60). Investigation of bile samples from rabbits injected with rose bengal, suggested that there is neither conjugation nor other forms of transformation of the dye in its passage through the liver (38,39). Only in exceptional cases, like severe liver deficiency, intra and extrahepatic jaundice,

would small amounts of radioactive labeled rose bengal be found accumulated in the kidney or excreted in the urine (40,41,42,43). Other studies also reported that rose bengal did not pass through the GI epithelium in significant amounts nor was there any appreciable re-absorption from the intestine after being excreted through the bile (41,44). Today, this tracer is used primarily to detect and evaluate hepato-biliary disease (45), and with the introduction of the better radio-nuclide I-123, an improved detection and evaluation especially in hepato-biliary imaging in patients can be obtained (46).

5. Tc-99m Sulfur Colloid

When colloidal particles such as the Tc-99m sulfur colloid of 0.1 μ to 1 μ size are injected intravenously, they are rapidly cleared from the blood, principally by means of phagocytosis by the Kupffer cells of the hepatic system, and to a lesser extent by the spleen and bone marrow. Marked impairment of the function of the hepatic Kupffer cells or of the portal circulation delays this clearance and therefore results in progressively greater uptake of the colloid by other portions of the reticuloendothelial system, mainly the spleen and bone marrow (61, 62,78). While most of the colloidal particles are too small to be trapped in the capillary bed of the lungs, uptake in the lungs may be observed occasionally. This effect is usually related to the patient's disease such as impaired hepatic function (79), malignant lymphoma (84), histiocytosis (80), mucopolysaccharioeses (81), falcaparum malaria (82), and intravascular coagulation problem (83), rather than to such technical factors as macroaggregation of the radiopharmaceutical before injection. Migration and embolization of macrophages to the lung was suggested to be a possible mechanism for colloid uptake in the lung (86). No uptake is normally seen in the kidney. By monitoring the urine for 24 hours after the intravenous adminstration of Tc-99m sulfur colloid, Patton et al.have demonstrated that less than 0.2% of the radioactivity is excreted through the

kidneys (85). Conditions like renal transplant rejection (87), congestive heart failure (88), pleural effusion and ascites (89) have been reported to cause an increased renal uptake of the colloid. Factors like intravascular coagulation with entrapment of fibrin deposits in the renal capillaries (83), and the accumulation of histiocytes at sites of inflammation and necrosis (90), have been suggested as the possible mechanism. Having a safer and more convenient method of producing labelled sulfur colloid based on the reaction of pertechnetate ion with thiosulfate and gelatin as a stabilizer (63), Tc-99m Sulfur Colloid has gained widespread use as a diagnostic aid in determining the size, shape, position and functional integrity of the liver. The radionuclide has a 140 Kev gamma radiation, no beta emission, physical half life 6.02 hr., thus offers a relatively low radiation exposure to patients while yielding a high photon flux (64). Besides providing valuable information regarding the function and integrity of the liver in a simple and relatively non-invasive diagnostic procedure, malposition and displacement of the liver (69), diffuse parenchymal lesions of the liver particularly cirrhosis, fatty infiltration, acute and subacute hepatitis (65), hepatic metastasis, primary tumors, abscess (66,67,68), blood pool scans in hepatic veno-occlusive disease (75), assessment of liver regeneration (77), as well as a guide for needle or open biopsy can be provided with the use of Tc-99m Sulfur Colloid. Areas such as labeling of

phagocytes from human blood (70), bone marrow scanning (71), clinical correlation of hepatic flow studies (72), diagnosis of vertebral compression fractures (73), liver scan in Budd-Chiari Syndrome (74), imaging in splenogonadal fusion (76), and others, are under investigation.

6. Mono, di and tri radio-iodinated Hippuric Acid.

Radioactive iodine labeled hippurate or hippuric acid has been employed successfully in both clinical and research studies of some parameters of renal function (100,101, 102, 103). Measurement of effective renal plasma flow and urine flow fraction have been obtained by the application of a kinetic model of o-iodo-hippurate distribution and renal clearance. Modern computer-aid in the adjustment of variable parameters of a mathematical description of hippurate turnover in the body to extract quantitative clinically useful data from the conventional renogram offer considerable value for the diagnosis of major renal artery occlusion, bilateral renal vein thrombosis, obstruction due to stone, acute and chronic disorders of renal function and for evaluation of the therapeutic responses (104). Moreover, comprehensive evaluation of renal function in the transplanted kidney can be made available by the analysis of ortho-iodohippurate kinetics. Clinical entities like normally functioning transplanted kidney, acute tubular necrosis, cell-mediated rejection, humoral (chronic) rejection, and post-renal obstruction can be predicted as much as a week before manifestations by other techniques (105). Recently, with the improvement of sequential scintillation imaging and scanning techniques, radioactive iodohippurate has also been used as an imaging agent in the evaluation of renal transplant status (106) as well as to

visualize kidneys in the presence of renal impairment which could not be demonstrated with radioactive chlormerodrin scans (107).

Ortho-iodohippurate is rapidly removed from the blood and accumulated by renal tissue, the peak of activity occurring within 3 to 6 minutes in normal kidneys and over 6 minutes in abnormal kidneys (101). The radio-iodo-hippurate has the advantage of not being concentrated by the liver, thereby providing a sensitive measurement of kidney function (102). With a plasma concentration of less than 10 mg%, 20% of the iodo-hippurate is excreted by glomerular filtration and 80% by tubular secretion (103). Normal kidneys are reported to clear 50% to 60% of the radioisotope labelled hippurate within 20 minutes, 80% or more within 90 minutes (102). About 90% to 95% of the tracer is normally recovered from the urine within 8 hours after intravenous injection (102). There is a good correlation between the renal uptake of mono-iodohippurate, mono-iodohippurate clearance and renal function (101,102,103,106). Close correlation between renal blood flow and renogram parameters is also established (108).

R. T. Williams in his study with C-14 labelled benzoic acid which is metabolized into hippuric acid and benzoyglucuronide, reported that neither benzoic acid nor hippuric acid were excreted in the bile in more than trace

quantities after injection into rats (109). However, on tying the renal pedicle, 7.2% of ^{14}C-benzoic acid appeared in the bile in five hours, mainly as hippuric acid and benzoylglucouronide (109). Tying of the renal pedicle to prevent urinary excretion of benzoic acid which is known to be excreted mainly through the kidneys, caused small amounts of conjugated benzoic acid to appear in the bile, suggesting another possible alternate route for "drug detoxification" of this substance.

Williams and his colleagues (109) also went on to study the effect of increasing molecular size on the excretion of drugs, using iodine substituted Ortho- and P-aminobenzoic acids. The unsubstituted acids were observed to be excreted in the bile in small amounts (two and a half percent of the dose) and most of the biliary material was in some conjugated form. However, the iodo derivatives appeared in increasing amounts; the mono-iodo derivative of P-aminobenzoic acid at about 11 percent of the dose and the di-iodo derivative at a relatively higher percentage , in a period of 24 hours. A similar study with anthranilic acid showed 5.3% of the dose appeared in bile as metabolites in a period of 24 hours, but when the di-iodoanthranilic acid was used, about 35% of the dose was excreted through the bile. These experiments suggested that molecular size played an important role upon the route of excretion (109).

III Experimental Materials and Methods

1. Induction of experimental uremia in animals.

Male Wistar rats, weighing 175-200 gm at the onset of the experiments were obtained from Woodlyn Farms Ltd, (Guelph, Ontario). The rats were divided into control and test groups by random selection. In the test group, uremia was surgically induced by a two stage operation (3). The first one was a unilateral sub-total nephrectomy on the left kidney, removing 2/3 of the renal mass. The second one consisted of a contralateral total nephrectomy of the right kidney ten days later. A summary of the surgical procedures used are as following :-

1. Under light ether anesthesia, the rat was weighed and the surgical site was shaved and later swabbed with ethanol. The animal was then immobilized in a supine position on a dissecting board and further anesthetized with Penthrane® (methoxy-flurane NF).

2. A straight abdominal incision about one inch long was made through the skin from the sternum toward the navel.

3. The abdominal muscle layer was penetrated, and by careful blunt dissection, the right kidney was isolated from the connective tissue (renal fascia)

and the surrounding cushion of fat without damage to the adrenal gland.

4. The ureter, renal artery and vein were clamped by surgical clamp.

5. Two-thirds (upper and lower poles) of the kidney was excised. Surgicel® (oxidized regenerated cellulose) was stitched to the exposed renal mass using 4-0 chromic catgut to effect hemostasis and to promote healing.

6. The clamp was removed and the kidney was placed back into the abdominal cavity.

7. The abdominal wall was closed with running 4-0 chromic catgut sutures, and the skin was approximated with 11 mm metal clamps.

8. The second operation was performed ten days later in each animal, and consisted the same isolation technique on the right kidney through the incision at the right dorsal and inferior part beneath the rib cage. The ureter and blood supply were ligated and the entire kidney was excised and removed. The peritoneal wall and skin were sutured as previously described.

Both control and uremic rats were maintained on Purina® Rat Chow. They were individually housed in wire cages, and kept in a room at constant temperature (22°) with twelve hours of light per day. Food and water are allowed ad lib. Experiments begun 60 days after surgery to allow for the development of chronic renal failure. Normal rats of the same age as the rats with renal failure were used for control studies.

During this 60-day period, several signs and symptoms were observed in the operated animals. They seemed to be lazy, fatiqued, exhibited a decrease in mental alertness, anorexia, anemia, and the fur developed a slight yellow coloration in comparison with the normal ones. Serum calcium levels were recorded to be slightly lower than those in the normal rats close to the end of the 60 day period.

2. Serum Calcium Determination By Atomic Absorption Spectrophotometry

Changes of several clinical parameters such as the elevation of BUN and creatinine levels in the blood, along with progression of the renal disease have been well documented. The chronic stage of renal failure is usually associated with abnormal calcium metabolism (25,28). The development of hypocalcemia is primarily due to the defective absorption of calcium from the intestine (25, 26, 27). These are the result of Vitamin D resistance and the abnormality of Vitamin D metabolism secondary to a combination of factors including hyperphosphotemia and secondary hyperparathyrodism (26, 29, 30). Slightly lower serum calcium level in the chronic renal failure animal model compaired to the controls has also been observed (10).

Serum calcium level was determined by Atomic Absorption Spectrophotometry using a Perkin-Elmer 290B Atomic Absorption Spectrophometer, calcium hollow cathode lamp provided by Perkin-Elmer as the emitter source, Beckman Standard Calcium Solution (Salt $CaCl_2$, 10 meq/l.) as source for calcium standard solution preparation and Acetylene/Air mixture as the burning fuel. Double distilled water was used as the primary solvent throughout the procedure and all glassware was

soaked in 1 N HCl in double distilled water overnight and rinsed several times with doubled distilled water prior to use in order to remove traces of calcium in glasswares. The working diluent employed was 0.5% LaCl3, 6% butanol in 0.1 N HCl for both standards and serum sample (31). Calcium standards with concentration of 4 mg/ml, 10 mg/ml, 16 mg/ml and 20 mg/ml were employed. A 0.25 ml of serum sample from the animal just prior to the tracer study was diluted exactly to 2.5 ml with the working diluent and the reading recorded by atomic absorption spectrophotometry was compared to the standard curve produced by the calcium standards. The values obtained were expressed as calcium level in mg% (31).

3. Radio-isotopes use

a. Iodine-125

Iodine-125 was the isotope of choice for the labeling of rose bengal, mono, di, and tri-iodohippuric acid. It has a half life of 60.2 days and offers the possibility of a longer period of time for research manipulation while the correction of radioactivity decay during the in vivo detection is minimum. For small animals like the rats we employed for both the normal and renal failure models, the 35 keV gamma radiation offered by Iodine-125 can be detected externally without difficulty by scintillation crystal detector with window setting corresponding to the 35 keV gamma radiation. Iodine-125 is readily available commercially in carrier-free form as I-125 sodium iodide solution.

b. Technetium-99m

Technetium-99m decays by isomeric transition with a physical half-life of 6 hours. As one of its principal radiation emissions consists of a gamma ray of 140.5 keV energy, it is very useful for scanning and imaging studies. It is also readily available from Molybdenum -99-Technetium-99m generators as a carrier-free Tc-99m sodium pertechnetate solution. The sulfur colloid used in this study was labeled with Technetium-99m for liver

investigation.

4. Chemicals and Reagents

Chemicals:-

All chemicals and reagents used in this investigation were of A.C.S. Or analytical grade quality.

Rose Bengal (Fisher Scientific)

p-iodobenzoic acid (Eastman Organic Chemicals)

p-aminohippuric acid (Aldrich Chemical)

3,4,5 -triiodobenzoic acid (Aldrich Chemical)

Chromatograms:-

Whatman No.1 Chromatography Paper (Whatman)

Eastman Chromagram Sheet with fluorescent indicator (Kodak)

Chromatographic Column:-

Sephadex LH-20 column (Pharmacia)

Radioactive Isotopes:-

Radioactive iodide I-125 was obtained from New England Nuclear, Boston, Mass.U.S.A. in the form of carrier-free I-125 sodium iodide. Specific activities of 20 mCl/ml were received in 0.5 ml volume of 0.1 M NaOH.

5. Preparation of I-125 Rose Bengal (125)

a. Chemical Separation and Labelling.

I-125 Rose Bengal was prepared by exchange labelling of the major fraction of commercial rose bengal (Fisher Scientific, Edmonton, Alberta) with carrier free NaI-125 (New England Nuclear, Boston, Mass.) (48, 49). A saturated solution of rose bengal in ethanol was passed through a millipore filter to remove insoluble particles, then the major fraction was isolated by chromatography on Sephadex LH-20 column (Pharmacia, Montreal, Quebec) with ethanol:chloroform (3:2) as the eluting solvent. Several components were seperated and as listed from fastest moving component to the slowest one were: R1 (orange), R2 (pink), R3 (red), R4 (clear) and R5 (purple). R3 was the major fraction and contained the majority of the dye. The entire chromatographic process took about 24 hours. Iodine-125 was introduced into the molecule by reflux heating (65-75°C, 8 hours) the major fraction (3 mg) and $Na^{125}I$ (3 mCi) in sodium acetate buffer (1M, 5 ml, pH 4.). The radio-labeled product was isolated and purified by repeated precipitation and dissolution cycles in 5 M HCl and 1 M NaOH respectively. The final product was dissolved in 1M NaOH, the pH adjusted to 7.0 with 1 M phosphate buffer using a glass electrode pH meter, and finally passed through a 0.45μ Millipore filter.

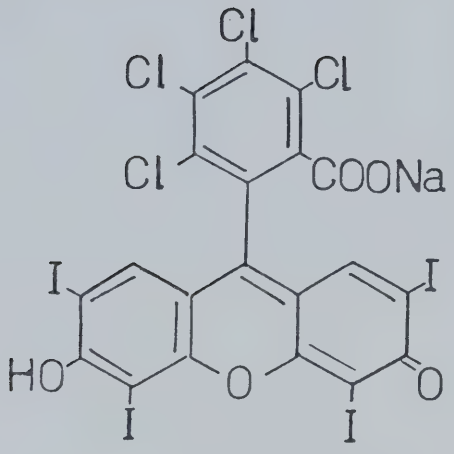


Figure 1. Rose Bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein sodium)

b. Radiochemical purity and specific activity assay.

Radio-chemical purity of the final preparation was greater than 95%, as determined by ascending chromatography on Whatman No. 1 paper using 5% NH3:25% Ethanol:Butanol (1:1:1) as eluting solvent system and assay by a linear gamma scintillation chromatogram scanner. Radioactive assay of final product was carried out by using a well-type scintillation counter (Autowell II). The specific activity was 42.2 μCi mg^{-1}; the dose per animal was 4.2 μCi.

6. Preparation of Tc-99m Sulfur Colloid

Freshly prepared Tc-99m sulfur colloid with a specific activity of 20 μ Ci per 0.1 ml was obtained from the Department of Nuclear Medicine, W. W. Cross Cancer Institute, Edmonton, Alberta. The radiochemical purity of all the batches was routinely greater than 95% as determined by thin layer chromatography (Gelman ITLC S.G.) with 0.9% sodium chloride as solvent. The formulation of the Tc-99m sulfur colloid consisted of a solution of sodium thiosulfate and sodium perrhenate in sterile water for injection, a solution of 1N hydrochloric acid and a solution of 0.3M phosphate buffer.

7. Preparation of radio-iodinated hippuric acids

A number of syntheses and labeling techniques were studied (110,111,112) for the preparation of para-iodohippuric acid, 3,5-diiodohippuric acid and the 3,4,5-triiodohippuric acid. All of these were first prepared chemically and then labelled with the radioactive Iodine-125 by a direct exchange method.

A. Preparation of p-iodohippuric acid.

One gram of pure p-iodobenzoic acid in anhydrous diethyl ether was refluxed gently with 3.5 ml redistilled thionyl chloride for 2 hours along with constant gentle stirring. The ether and the thionyl chloride were distilled off on a water bath. At the same time, 0.5 gm of glycine was dissolved in 5 ml of 10% sodium hydroxide in absolute ethanol contained in a wide-mouthed bottle. The p-iodobenzoyl chloride obtained was then added in five portions to the solution with vigorous and continuous stirring with a magnetic stirrer until all the chloride was reacted. Then the solution was transferred to a beaker along with the absolute ethanol rinsing. A small amount of crushed ice was added into the solution and immediately pre-cooled concentrated hydrochloric acid (HCl) was added slowly with stirring until the mixture was acidic to pH paper. The resulting crystalline p-iodohippuric acid which was

contaminated with a trace of benzoic acid, was collected. The solid was washed with a small amount of cold water and filtered in a Buchner funnel. The solid was then placed in a small beaker with 3 ml of carbon tetrachloride, covered with a small watch glass and gently boiled for 10 minutes. Traces of benzoic acid present were extracted by the carbon tetrachloride. The mixture was allowed to cool slightly, then filtered by gentle suction and the P-iodohippuric acid was washed again with 2 ml of carbon tetrachloride on the filter. After boiling with about 10 ml of water, the pure p-iodohippuric acid was allowed to recrystallize and the purified p-iodohippuric acid was then collected in a Buchner funnel and later dried (112). The yield of p-iodohippuric acid was about 65% compared to the original p-iodobenzoic acid used.

Thin layer ascending chromatography using Eastman Chromagram sheet (with fluorescent indicator) and solvent systems like ether:hexane(1 : 9), butan-1-ol:acetic acid:water (12 : 3 : 5) were used to identify the formation of p-iodobenzyol chloride from p-iodobenzoic acid (113). The p-iodobenzoic acid, intermediate product and the formation of p-iodohippuric acid were double checked with ascending paper chromatography on Whatman No. 1 paper and solvent system n-butanol:water:glacial acetic acid (120:50:30) (110). The final purity of P-iodohippuric acid was checked by the Nuclear Magnetic Resonance Spectroscopy.

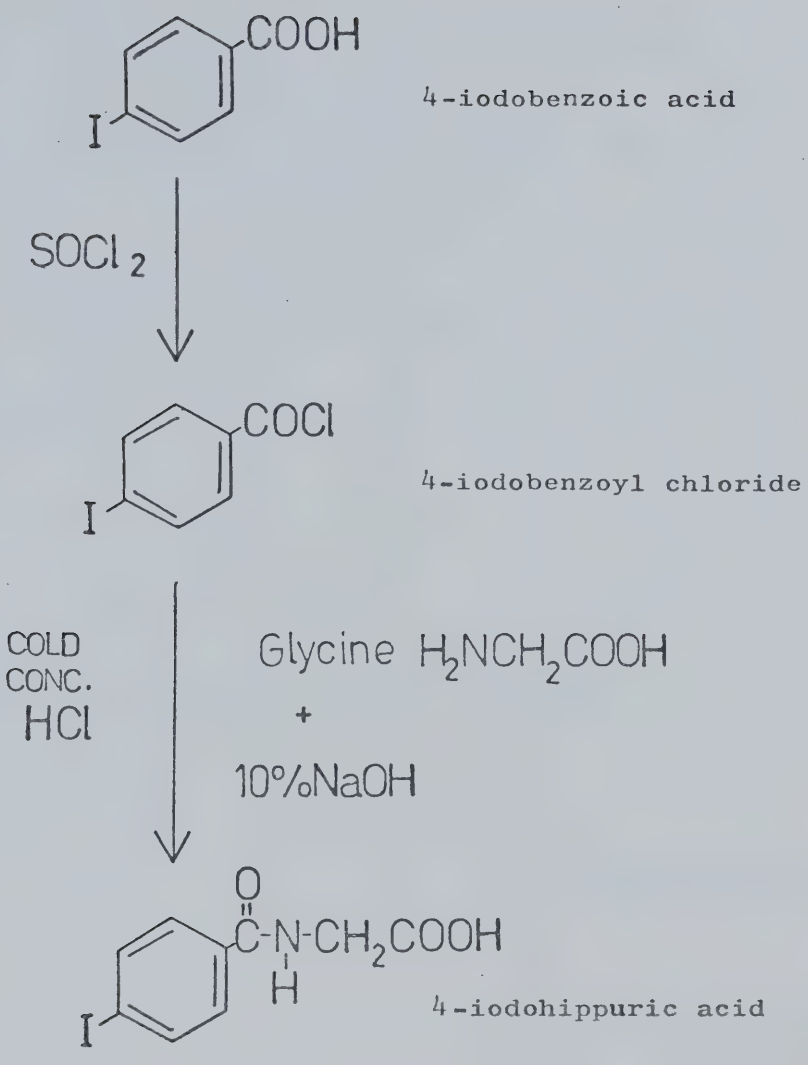


Figure 2. Chemical preparation of 4-iodohippuric acid.

B. Preparation of 3,5-diiodohippuric acid.

10 gm of the pure p-aminohippuric acid was dissolved in 450 ml of warm (75°) 12.5% hydrochloric acid in a beaker provided with a mechanical stirrer. A solution of 48 gm of iodine monochloride in 40 ml of 25% hydrochloric acid was added and the mixture was stirred for one minute. During this time, a brown precipitate appeared. The reaction mixture was then diluted with 1 litre of water whereupon a copious precipitate was deposited. The temperature of the well-stirred mixture was raised gradually and then maintained at 90° for 15 minutes. Then it was allowed to cool to room temperature, filtered, washed throughly with water and then air dried. The crude acid was then purified by dissolving it in dilute sodium hydroxide and then precipitating with dilute hydrochloric acid followed by filtration and drying (112). The yield of 4-amino- 3,5-diiodohippuric acid was about 85% of the p-aminohippuric acid used.

3.4 gm of this 4-amino- 3,5-diiodohippuric acid was dissolved in 15 ml of cold concentrated sulphuric acid, and a large excess (1.5 gm) of powdered sodium nitrite was added and the mixture was allowed to stand at -5°C for 2 hours. Then the cold diazonium solution was treated with 20 ml of cold 50% hypophosphorous acid which was added over a period of 10 to 15 minutes, and a precipitate was separated. The

mixture was then warmed on a water bath until the evolution of nitrogen ceased. The precipitate of crude 3,5-diiodohippuric acid was then purified by recrystallization from dilute alcohol (112,114,115,116). The molar yield of 3,5-diiodohippuric acid to the amount of 4-amino- 3,5-diiodohippuric acid was about 80%.

The intermediate product, the final 3,5-diiodohippuric acid and its purity were checked with Nuclear Magnetic Resonance Spectroscopy.

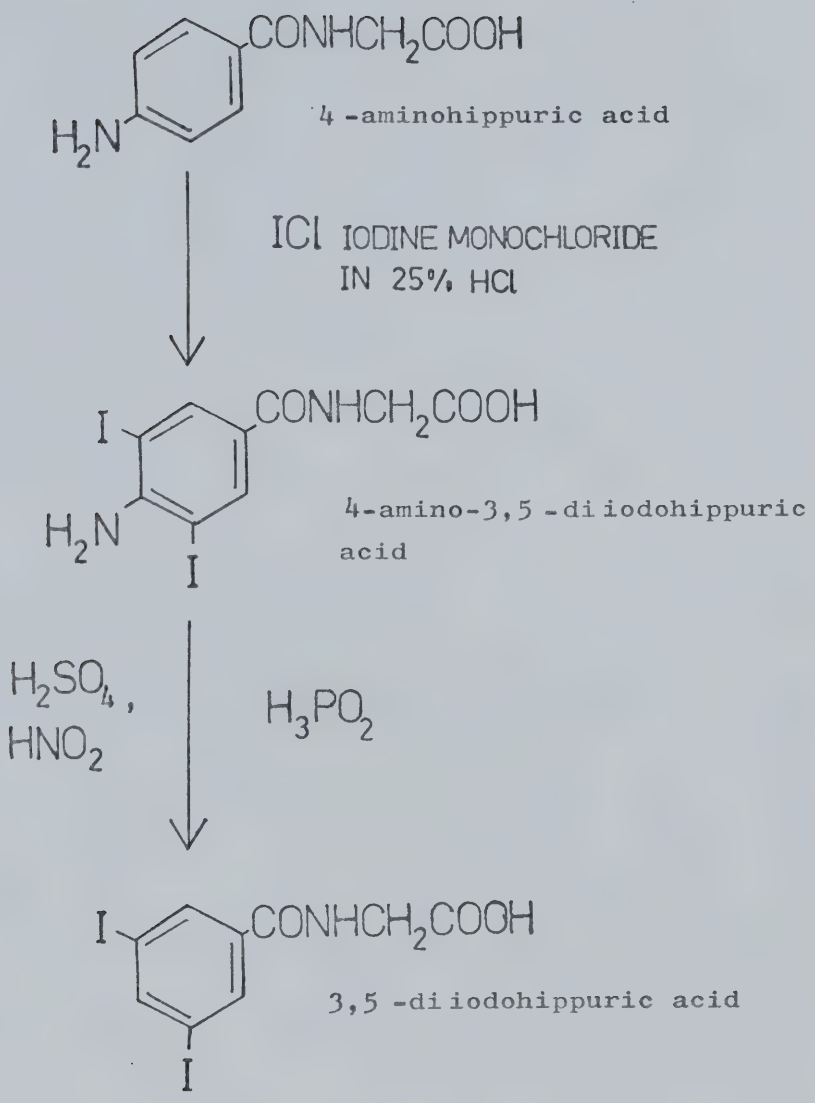


Figure 3. Chemical preparation of 3,5-diiodohippuric acid.

C. Preparation of 3,4,5-triiodohippuric acid.

2 gm of pure 3,4,5-triiodobenzoic acid in anhydrous ether was refluxed gently with 3.5 ml redistilled thionyl chloride for 2 hours along with constant gentle stirring. The ether and the excess thionyl chloride were distilled off on a water bath. 3,4,5-triiodobenzoyl chloride was formed at this stage. At the same time, 0.5 gm of glycine was dissolved in 5 ml of 10% sodium hydroxide in absolute ethanol containing in a wide-mouthed flask. The 3,4,5-triiodobenzoyl chloride obtained was then added in five portions to the solution with vigorous stirring with a magnetic stirrer until all the chloride was reacted. Then the solution was transferred to a beaker along with absolute ethanol rinsing. Then, a small amount of crushed dry ice was added into the solution and immediately pre-cooled concentrated hydrochloric acid (HCl) was added slowly and with stirring until the mixture was acid to pH paper. The resulting crystalline precipitate of 3,4,5-triiodohippuric acid along with small amount of remaining 3,4,5-triiodobenzoic acid, was collected (112). The solid was washed with a small amount of cold water and well drained upon a Buchner funnel. The solid was then placed in a small beaker with 3 ml of carbon tetrachloride, covered with a small watch glass and slowly boiled for 10 minutes. Traces of 3,4,5-triiodobenzoic acid which were present were extracted by the carbon tetrachloride. The mixture was

allowed to cool slightly, then filtered by gentle suction and the 3,4,5-triiodohippuric acid was washed again with 2 ml of cold carbon tetrachloride on the filter (112). After dissolving in about 15 ml acetone and water (1:1) and stirred for 5 minutes, the pure 3,4,5-triiodohippuric acid was precipitated by concentrated HCl acid added slowly dropwise. The pure 3,4,5-triiodohippuric acid was then collected in a Buchner funnel, washed with small amount of distilled water, then dried. The yield of 3,4,5-triiodohippuric acid was about 60% compared to the original 3,4,5-triiodobenzoic acid used.

Thin layer ascending chromatography using Eastman Chromagram sheet (with fluorescent indicator) and different solvent system like ether : hexane (1:9), ethyl acetate:acetone (1:1), chloroform:acetic acid (9:1) and n-butanol:acetic acid : water (4:1:1) (113,117) were used to identify the formation of 3,4,5-triiodohippuric acid from the 3,4,5-triiodobenzoic acid and any intermediate compound. Upon examination under short wave UV light, all products appeared as brown spots on the chromatogram. However, under long wave UV light, only 3,4,5 tri-iodobenzoic acid appeared as a blue spot. The identification of the final product and its purity was further checked with Nuclear Magnetic Resonance Spectroscopy.

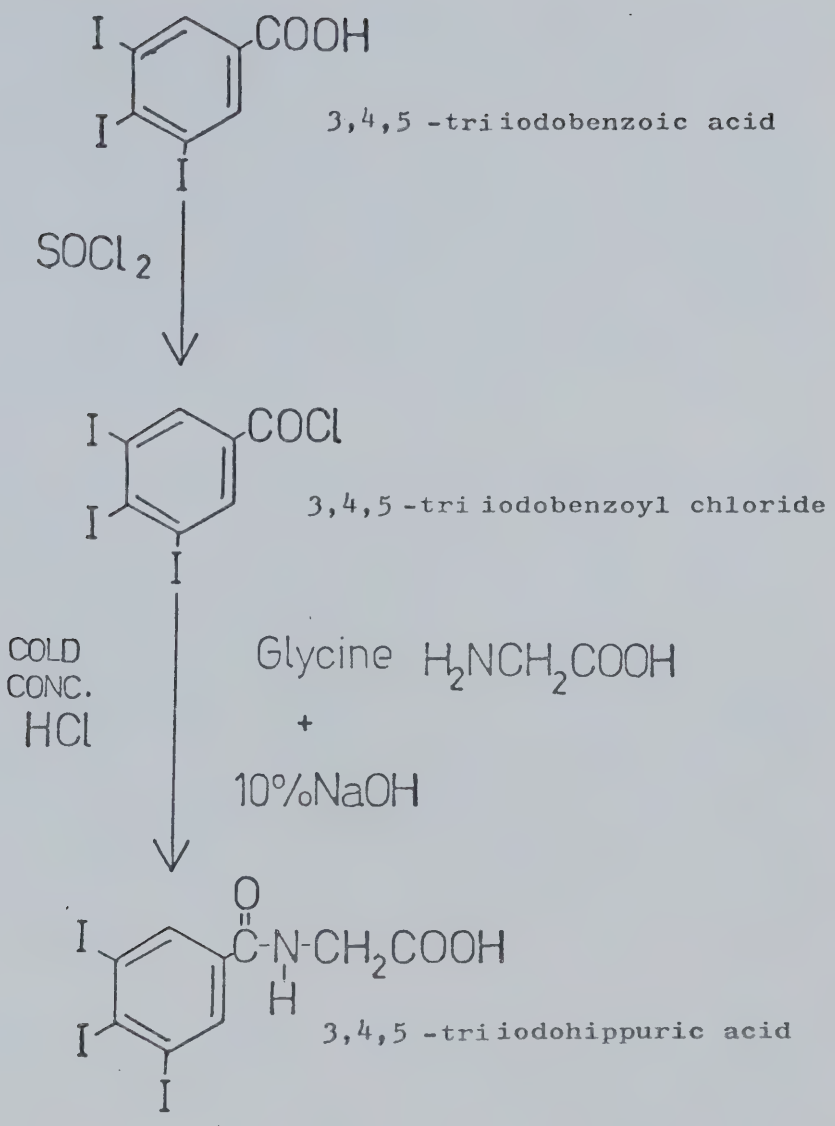


Figure 4. Chemical preparation of 3,4,5-triiodo-hippuric acid.

D. I-125 Labelling and Radiochemical Purity assay.

The mono-, di- and tri-iodohippuric acids prepared have molecular weights of 305, 432 and 559 respectively. When they were labelled with I-125 instead of I-127 (normal stable isotope), the molecular weights were then 303, 428, and 553 respectively. Since the final injection products were in the form of sodium hippurate, the molecular weights of each compound would be increased by 23.

Mono-, di-, and tri-iodohippuric acid were labeled with radioactive I-125 by the direct exchange method. One ml of 0.01 M solution of the particular non-radioactive hippuric acid and 1 mCi of sodium iodide-I-125 (carrier-free), were heated at 60° - 80°C for 6 hours in 1 M sodium acetate buffer at PH 5.5. The labelled product was precipitated using 5 M HCl. The cycle of precipitation and dissolution was repeated until the product was free from free I-125 contamination (118).

The product was finally dissolved in dilute NaOH, adjusted to pH 6-8, made isotonic and aseptically filtered through a 0.45u millipore filter into a small sterile multidose vial and stored in a refrigerator (118).

Radioactivity assay was carried out using a well type ionization dose calibrator, and a Picker Autowell II well-

type gamma scintillation spectrometer. The pH was determined by glass electrode. Radiochemical purity was determined by ascending paper chromatography using Whatman No. 1 paper and a solvent system with n-butanol : acetic acid : water (4:1:1), pH 2.4. The development time was about 3 hours and the Rf for Iodide was around 0.09 while the Rf for hippurate was around 0.85 (119). Radiochemical purity assay for unbound iodide-125 activity in the final injections were rating less than 5% as determined by a gamma scintillation linear chromatography scanner.

8. Investigation Procedures.

A. I-125 Rose Bengal Excretion Study.

The chronic renal failure model was prepared surgically by sub-total nephrectomy of male Wistar rats as described previously (21,22, 23, 47). Liver function studies were conducted sixty days following the second (contralateral nephrectomy) surgical procedure. Normal rats of the same age as the renal failure rats were used in control studies.

Clearance of I-125 rose bengal from circulating blood, and excretion via the bile, was determined for both normal and uremic animals which had been given drinking water containing Lugols Solution (0.1 ml l^{-1}) for ten days prior to the study in order to block the thyroid uptake of possible traces of free I-125 which might interfere with the blood clearance measurement. Test animal were anesthetized by i.p. injection of urethane (0.5 ml Kg^{-1}; 25% w/v aqueous solution).

Bile was collected with a polyethylene cannula inserted into the common bile duct through a mid-line abdominal incision. For clearance studies, the cannulated, anesthetized rat was immobilized in the supine position. A 19 mm diameter sodium iodide scintillation crystal was installed in a 9mm thick cylindrical lead collimator with the detector surface withdrawn (25 mm) from the open end of

the collimator. With this detection assembly placed directly above the left jugular vein it was possible to monitor the I-125 levels in the circulating blood with no interference from radioactivity in other organs. Those events corresponding to the 35 Kev emissions of I-125 were stored in a Northern Scientific model 636 multichannel analyser (Northern Scientific, Middleton, Wis.) operating in the multichannel scaling mode. This raw data describing the variation of I-125 activity in the blood with time was temporarily stored with a Dicom model 344 cassette magnetic tape system (Dicom Industries, Sunnyvale, Calif.).

B. Tc-99m Sulfur Colloid Uptake Study.

The chronic renal failure animal model was prepared surgically by sub-total nephrectomy of male Wistar rats. Normal rats of the same age were used in control studies. Serum calcium levels were determined in both groups by Atomic Absorption Spectrometry as described previously.

20μCi of Tc-99m sulfur colloid in a 0.1 ml volume, was injected iv. into each animal via the femoral vein. Clearance of Tc-99m sulfur colloid from the circulating blood and the uptake rate in the liver were monitored by two 19mm diameter sodium iodide scintillation crystals which were installed in two 9mm thick cylindical lead collimator with the detector surface withdrawn (25mm) from the open end of the collimator. One detection assembly was placed directly above the left jugular vein so that it was possible to monitor the Tc-99m sulfur colloid level in the circulating blood with little interference from radioactivity in other organs. The other detection assembly was placed directly over the liver in the anterior position slightly to the right hand side so that the interference from the spleen and other organs could be minimized. Those events corresponding to the 140 Kev emissions of Tc-99m were stored in the Northern Scientific model 636 multichannel analyser (Northern Scientific, Middleton,Wis.) operating in the multichannel scaling mode. These raw data describing the variation of Tc-99m activity

in the blood and liver with time was temporarily stored with a Dicom model 344 cassette magnetic tape system (Dicom Industries, Sunnyvale, Calif.). Immediately following the 15-minute excretion study, the animals were sacrificed. A blood sample was collected and the whole liver and spleen were excised for measurement of radioactivity to indicate the level of Tc-99m Sulfur Colloid remaining in each organ system.

C. Mono- di- and tri- I-125 hippuric acid study

Lugols solution (0.1 ml l^{-1}) was added to the drinking for ten days prior to the *in vivo* dynamic studies of the radioiodinated hippuric acids. The animals (control or surgically induced renal failure models) were anesthetized with a 25% solution of urethane injected intraperitonally at a dosage of 0.5 ml per 100 gm body weight. Each animal was secured on a specially constructed platform in a supine position. Three 19 mm diameter sodium iodide scintillation crystal were installed in a 9 mm thick cylindrical lead collimator with the detector surface withdrawn (25mm) from the open end of the collimator in order to minimize interference and background radiation. The first detection assembly was placed directly above the left jugular vein to measure the radioactivity in the blood exclusively, the second detector was placed directly above the liver to measure the uptake and excretion of tracer in the liver and the third one was positioned at the posterior side of the animal aimed directly at the left side of the kidney in order to measure the uptake and excretion of tracer through the kidney concerned. The femoral vein was used for the injection of tracer which was 1/15 of 0.01 mM of that particular hippuric acid in a volume of 0.1 ml per i.v. dose; the specific activity was 3 to 4 microcuries per dose depending on the particular activity of that hippuric acid. The total scanning period was about 25 minutes per

animal. All these measurements were corresponding to the 35 Kev gamma emissions of I-125 and the data was stored in a Northern Scientific model 636 multichannel analyser operation in the multichannel scaling mode. These raw data were then transferred to the Dicom model 344 cassette magnetic tape system for further analysis.

Bile excreted during the scanning period was collected through a bile duct cannulation procedure in order to determine the total amount of radioactive tracer excreted from the liver through the bile. Blood samples were also collected to check the amount of radioactive tracer remaining in the blood circulation after the scanning period of 25 minutes.

IV. Results and Discussion of Results

A. I-125 Rose Bengal excretion study (125)

Results

Rate constants were calculated for the clearance of radioactivity from the blood of each animal. These values are given in Table I, together with the respective serum calcium levels and the total biliary elimination of I-125 rose bengal for each rat. Typical clearance curves for normal and uremic rats are shown in Figure 5.

Statistical comparisons of the means of the data from the two groups (Student t test) show that these parameters are significantly different in the animals with renal failure than in the normal group. The differences of blood clearance rate constants , serum calcium levels, and biliary elimination from normal values are significant at the 97.5%, 99.5%, and 99.8% confidence levels respectively.

Renal failure, however, is a complex syndrome which can, even in the surgically-induced model, assume a broad time-course relationship. That is, although animals were tested at a given time following sub-total nephretomy, the development of the syndrome varied from one animal to the next. A more reliable assessment of the impact of chronic renal failure on hepatic function can be realized by graphical comparison of any two parameters. Such comparisons

are presented in Figure 6.

Linear regression analysis of the combined data from all animals was undertaken. It was found that clearance rate constants expressed as a function of serum calcium, total biliary elimination as a function of serum calcium, and biliary elimination as a function of clearance rate constants, followed by linear correlation analysis gave correlation coefficients of 0.89, 0.86 and 0.92 respectively.

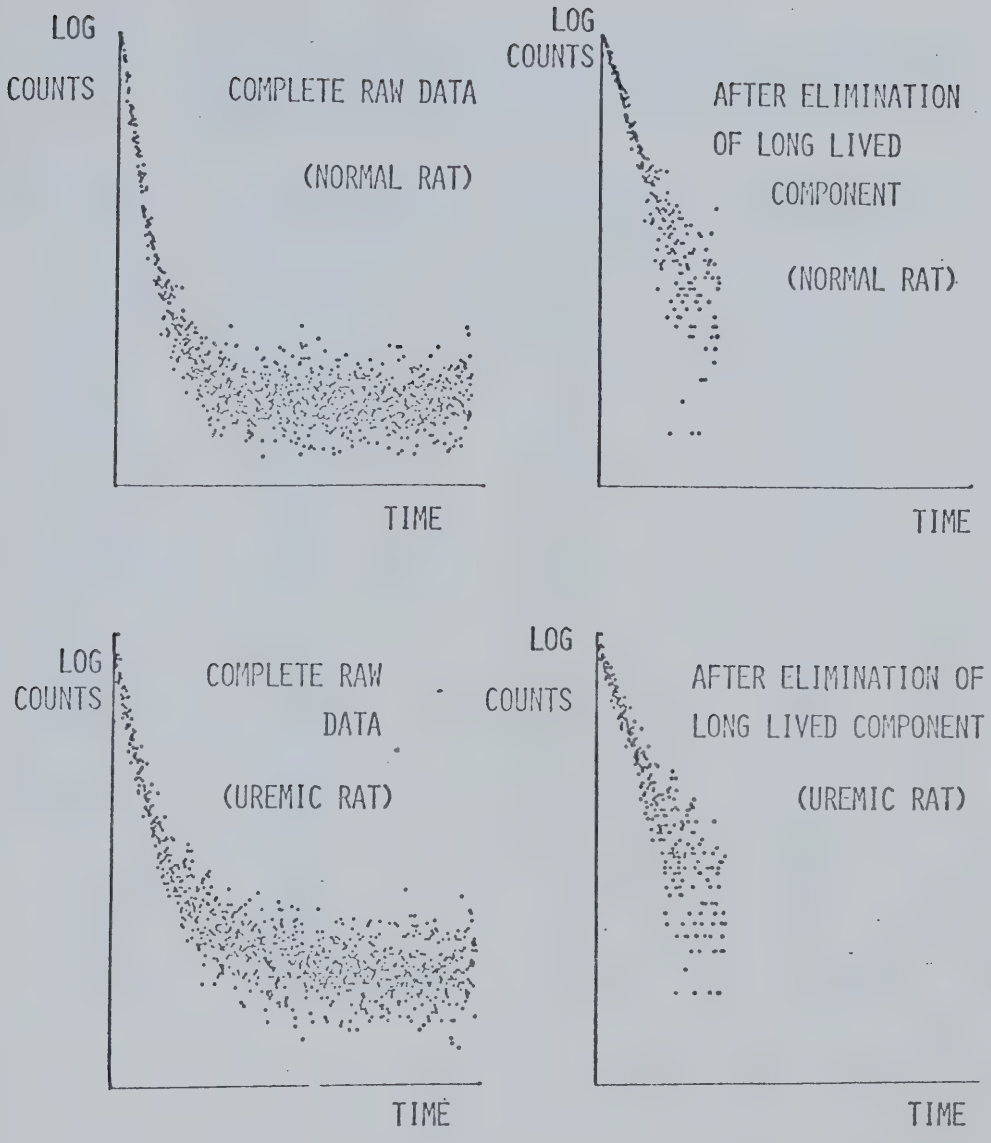


Figure 5. Blood clearance curves for ^{125}I-rose bengal, following intravenous injection into normal and uremic rats. The curves correspond to data presented in Table I for rats N-5 (normal) and U-4 (uremic) respectively.

Table I. Rate constants for clearance of ^{125}I-rose bengal from circulating blood, serum calcium levels, and total biliary elimination of ^{125}I-rose bengal following a single intravenous dose of ^{125}I-rose bengal.

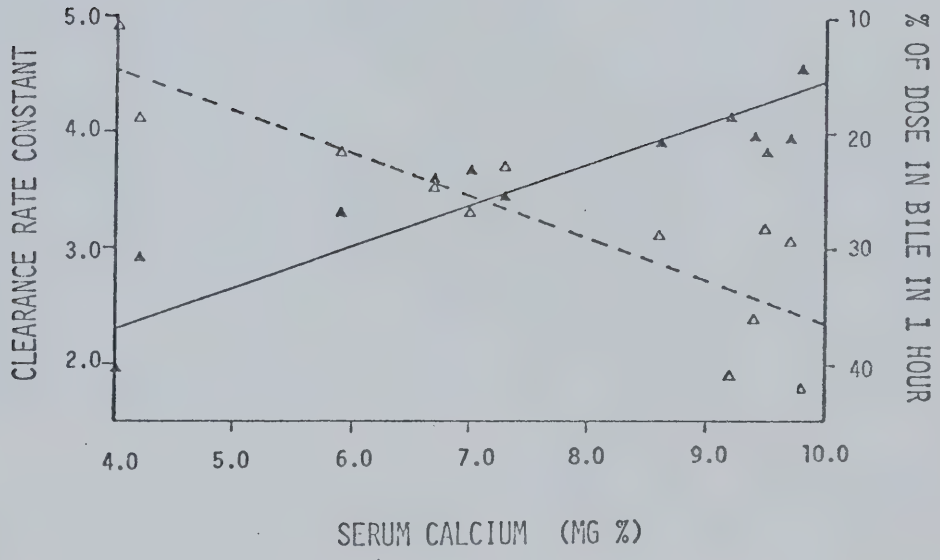
Animal	Clearance rate constant ($1x10^{-3}$)	Serum calcium (mg 100 ml^{-1})	Biliary elimination (% of dose)
N-1	4.52572	9.8	42.2
N-2	3.93587	9.7	28.9
N-3	3.82086	9.5	27.7
N-4	3.98333	9.4	35.8
N-5	4.10579	9.2	40.6
N-6	3.91006	8.6	28.8
N-$\bar{X}$ ± S.D.	4.0470 ± 0.2526	9.4 ± 0.4	34.0 ± 6.4
U-1	3.44512	7.3	22.7
U-2	3.70001	7.0	26.7
U-3	3.59874	6.7	24.2
U-4	3.29225	5.9	21.6
U-5	2.96862	4.2	18.9
U-6	1.90535	4.0	10.7
U-$\bar{X}$ ± S.D.	3.1517 ± 0.6623*	5.9 ± 1.4*	20.8 ± 5.6*

* $p \leq 0.05$

N (Normal Controls)

U (Uremic)

Figure 6. Clearance rate constants for ^{125}I-rose bengal (▲) and ^{125}I-rose bengal eliminated in bile in 1 hour (△), as a function of serum calcium levels for both normal rats and for chronically uremic rats. The calculated linear regression lines are (—) and (---) respectively.



Discussion of Results

The separation of commercial rose bengal into its various components has been found to be unnecessary for biliary elimination studies, in that several chromatographically separable fractions in rat bile appeared in approximately the same proportions as they occurred in the injected material (38), and that plasma decay curves in the rabbit were found to be the same for the multi-component commercial material and a pure component (39). However, some fractions have been found to remain unlabeled by exchange techniques (4-6) and it was therefore considered essential that in these experiments a single component of the commercial dye be used to avoid uncertainties in the interpretation of the data. LH-20 column chromatography was chosen because of its ease of use and speed, and its ability to separate a colorless fraction visible under U.V. light (50) from the major fraction isolated from commercial material by paper chromatography (38).

It has been determined previously (38) that blood radioactivity curves, in rats given I-131 rose bengal by intravenous injection, could be resolved into two exponential components. Each of the two parts of the curve also were present in rabbit serum studies, and each could be further resolved into exponential functions (39). In the present studies, the blood radioactivity curves were treated

as bi-exponential functions, even though the shape of the curve observed in the chronically uremic animals differed markedly from that observed for normal animals (Figure 1). This approach greatly simplified data analysis, without reducing the potential clinical imapct of the findings. The effects of other potential uremic changes such as altered plasma protein binding and proteinuria, have not been ascertained.

Visual inspection of the clearance curves depicted in Figure 1 may lead to an interpretation of the results which would suggest the absence of significant differences between groups of normal and chronically uremic rats. It would therefore appear essential that rigid mathematical analysis of data be taken in any evaluation of this type of work, to prevent oversight of significant information. In this case, the clearance rate constants of the two exponential components observed in the raw data were determined with the aid of a Digital Equipment PDP 11-05 minicomputer (Maynard, Mass.) interfaced to the NS636 multichannel analyser. The computer program written for this purpose performs a weighted least squares fit to the linear part of a plot of the logarithm of the observed count rate versus time. The contribution of the longer lived component is subtracted from the experimental data and the fitting process resumed to give the decay constant of the shorter lived exponential component. The program provides estimates of precision of

the decay constants as well as the relative amounts of the two components present. Figure 1 shows the blood clearance curves during the two stages of the fitting process. The decay constant of the shorter lived exponential component was used as the index of the blood clearance since the long lived component gave rise to levels of radioactivity which changed by only a small amount during the course of the experiment .

The average clearance rate constant of I-125 rose bengal from the blood in normal animals was 4.047×10^{-3} while the animals with renal failure was 3.152×10^{-3}. Biliary elimination during the experiment in the normal animals had a mean of 34.0% of the dose while the animals with renal failure was 28.8%. The results suggested that not only is the clearance of certain high molecular weight xenobiotics through the polygonal cells delayed in the animals with renal failure, but the overall elimination of these substances from the body will be delayed. It has been found that xenobiotics with unconjugated molecular weight of greater than 150 will undergo extensive blood clearance by the liver (51, 52). The importance of the observation of delayed clearance of such substances viz. rose bengal (MW 974) in this model of chronic renal failure may therefore, be of significance to the design and implementation of adequate drug therapy regimens clinically.

The range of chronic renal failure in the animals studied in this investigation deserves additional comments. It has been shown (53) that BUN is a poor predictor of the state of development of the chronic uremic syndrome in these sub-totally nephrectomized rats. However, in this model, cessation of skeletal mineralization has been demonstrated in animals which have been maintained BUN values in excess of 60 - 80 mg % for 6 - 8 weeks. The serum calcium level therefore might be an alternate parameter of metabolic derangement (10, 54).

B. Tc-99m Sulfur Colloid Uptake Study

Results

Rate constant for the clearance of Tc-99m sulfur colloid radioactivity from the blood and uptake into the liver of each animal were calculated. These values are given in the tables, together with the respective percentage activity captured in the liver and spleen, as well as the serum calcium levels. Statistical comparisons of the means of the data from the two groups (Student's t test) show that some parameters are significantly different in the animals with chronic renal failure than in the normal group. Liver uptake in chronic renal failure was 69.23% within 15 minutes, significantly different than the normal 55.75%. The mean liver uptake rate constants of 14.34×10^{-3} in chronic renal failure was significantly different from the values of 9.03×10^{-3} in normals. The respective blood clearance rate 10.81×10^{-3} and 7.51×10^{-3} were also significantly different from one another.

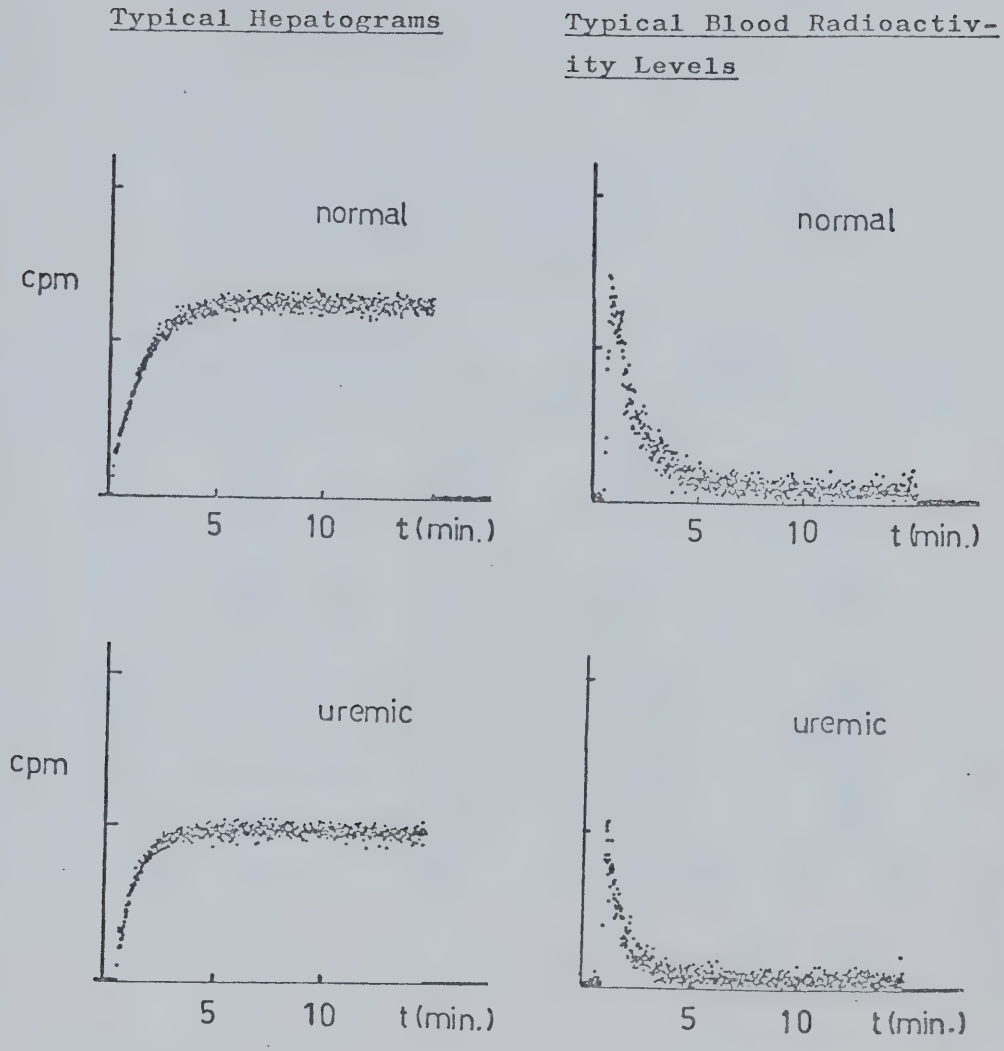


Figure 7. Typical hepatic uptake and typical blood radioactivity levels after i.v. administration of Tc-99m Sulfur Colloid to normal and uremic rats.

TABLE II Serum calcium levels, percentage weight of liver and spleen to the total body weight, and the radioactivity of Tc-99m Sulfur Colloid remained in liver and spleen 15 minutes after a single intravenous dose of Tc-99m Sulfur Colloid.

ANIMAL	SERUM CALCIUM	LIVER		SPLEEN	
	(mg 100 ml^{-1})	(% of Body)	(% of dose after 15 min.)	(% of Body)	(% of dose after 15 min.)
N-1	11.6	2.79	64.54	0.09	0.94
N-2	9.9	3.74	40.67	0.14	3.99
N-3	9.9	2.98	58.63	0.12	4.07
N-4	10.8	2.82	56.43	0.12	2.48
N-5	10.2	2.60	62.38	0.10	3.40
N-6	8.5	2.12	51.83	0.07	2.40
± S.D.	10.1 ± 1.0	2.84 ± 0.53	55.75 ± 8.63	0.11 ± 0.02	2.88 ± 1.18
U-1	7.1	3.05	66.22	0.15	1.44
U-2	8.1	3.21	77.40	0.15	4.26
U-3	8.5	4.00	63.47	0.19	2.21
U-4	8.2	4.10	64.57	0.12	3.19
U-5	5.8	5.94	68.40	0.22	2.10
U-6	7.1	3.22	75.34	0.25	2.08
± S.D.	7.5 ± 1.0*	3.92 ± 0.10	69.23 ± 5.80*	0.18±0.05*	2.55 ± 1.01

* $P \leq 0.01$

N (Normal Controls)

U (Uremic)

TABLE III Half uptake maximum time ($T\frac{1}{2}$), rate constants for liver uptake, and the clearance rate constants of Tc-99m Sulfur Colloid from circulating blood following a single intravenous dose of Tc-99m Sulfur Colloid

ANIMAL	LIVER UPTAKE ($T\frac{1}{2}$ in seconds)	LIVER UPTAKE Rate Constant (1×10^3)	BLOOD CLEARANCE Rate Constant (1×10^3)
N-1	94	7.37	6.02
N-2	84	8.25	7.68
N-3	64	10.83	9.09
N-4	74	9.37	7.76
N-5	82	8.45	6.78
N-6	70	9.90	7.78
$\bar{x} \pm$ S.D.	78 $\pm$ 10	9.03 $\pm$ 1.25	7.51 $\pm$ 1.04
U-1	58	11.95	10.14
U-2	50	13.86	9.65
U-3	50	13.86	11.67
U-4	48	16.16	12.13
U-5	44	15 75	11.74
U-6	48	14.44	9.50
$\bar{x} \pm$ S.D.	49 $\pm$ 5*	14.34 $\pm$ 1.51*	10.81 $\pm$ 1.17*

* $P \leq 0.01$

N (Normal Control)

U (Uremic)

Discussion of Results.

These results suggested that the animals with chronic renal failure have a faster clearance rate of Tc-99m sulfur colloid from the blood. The increase liver uptake rate constant suggested that the Kupffer cells of the liver in chronic renal failure had a higher capacity for retaining colloidal particles than the livers of normal rats.

There was no significant difference in the average percentage dose localized in the spleen. It was 2.55% for the chronic renal failure model compare to 2.88% in the normals. However, it was observed that the percentage weight of the liver and spleen to the total body weight was slightly increased in the chronic renal failure animal model. The mean liver weight to total body was 3.92% in chronic renal failure and 2.84% in normals while the spleen was 0.18% and 0.11% respectively. This may partially contribute to the increase in uptake of radiocolloid by these two organs.

Little or no information is available about the integrity of the phagocytic Kupffer cells during chronic renal failure nor the role they play in relieving the stress faced in uremic conditions. In the previous I-125 rose bengal study, chronic renal failure produced a general decrease of excretion of such a tracer which is normally

cleared by the polygonal cells in the liver. Interestingly, we observed an increase of Tc-99m sulfur colloid clearance from the blood by the phagocytic Kupffer cells in the liver during chronic renal failure. These observations suggested that the Kupffer cells are working quite independently to the polygonal cells, though they are both part of the integral hepatic system.

Kupffer cells of the liver , as part of the reticuloendothelial system (RES), consist of tissue macrophages originating from the circulating monocytes. The monocytes, like neutrophilic leukocytes, are actively phagocytic and contain peroxidase and lysosomal enzymes. They enter the circulation from the bone marrow, but after 24 hours they enter the tissue to become tissue macrophages. Rapid migration of monocytes and macrophages to the reticuloendothelial system or from the reticuloendothelial system to other organs such as lung, are in response to certain stress, chemotactic stimuli, inflammation, Vitamin B12, thyriod hormone, bacterial endotoxins, attenuated bacteria, foreign protein, and steroid hormones (86,91,92,93,94). The tissue macrophages (Kupffer cells in liver and sinusoidal lining cells of spleen) have also long been known to play a major role in malaria. Especially during the erythrocytic phase of the disease, the phagocytes of the RES served as primary scavengers removing the debris of the host-parasite interaction (95), and clinically, the

organs of the reticuloendothelial system were observed to become enlarged during the disease state. The spleen became palpable in 70 - 80% of such patients and hepatic enlargement occured in most (95). Histologically, this enlargement of the RES organs was primarily due to the tremendous hyperplasia and poliferation of macrophages, and these phagocytic cells became extremely active as the disease progressed (95). Studies measuring the clearance of colloidal particles have shown that these patients also associated with enhanced phagocytic activity of the RES (96). We do not know whether the similar physiological changes might exist in chronic renal failure, but the increase in Tc-99m sulfur colloid clearance rate constant from the blood, increase hepatic uptake rate constant, increase of percentage to the total dose of tracer entrapped in the excised liver, general slightly increased in size and weight of both liver and spleen observed, seems to suggest an increase in phagocytic activity of the RES in the liver, and probably associated with hyperplasia and proliferation of macrophages in animals with chronic renal failure.

Chronic renal failure is a very dynamic and complicated syndrome and no single parameter can directly assess the disease state nor is there one single treatment or therapy for chronic renal failure. Even under the stress and complication of uremia, some investigators have shown that there may be an increased rate of hepatic protein synthesis

in animal with renal failure in their studies (97). Giordano (98,99), also has proposed that uremic patients can reutilize urea nitrogen for the synthesis of non-essential amino acids. In this study using Tc-99m sulfur colloid , our result suggested a probable increase in the phagocytic activity of the Kupffer cells in the liver during chronic renal failure. These pieces of information and studies might be of significant in our further understanding of chronic renal failure and possible enrichment to our method, care, and therapeutic regiment in the treatment of chronic renal failure.

C. Mono, Di, and Tri-Iodohippuric Acid Study.

Results.

The model of chronic renal failure was characterized by altered hepatic weight, and decreased serum calcium levels in addition to the qualitative changes observed in the rat following surgery. This data, and the renal mass data for all uremic and control rats used in this investigation are presented in Table Ⅲ.

Radioactivity levels in serum, blood cells, bile, whole liver and total renal mass at termination of each experiment were measured (Table Ⅳ) for all animals involved. Blood level measurements were based on a 5 ml aliquot of whole blood which was allowed to clot and then contrifuged to separate cells from the serum. The bile data reflect the total activity collected from the biliary canula.

The renograms and hepatograms obtained were analysed to determine the time at which the organ displayed maximal radioactivity levels and to determine radioactivity elimination rate constants for the liver and kidney. The blood clearance rate constant was also calculated from the blood radioactivity data. Calculated values are given in Table . Typical blood radioactivity curves, hepatograms, and renograms respectively in normal and uremic rats after

dosing with different tracers are shown.

Figure 8 . Typical blood radioactivity levels after i.v. administration of radioiodinated 4-iodo,3,5-diiodo-,and 3,4,5-triiodohippurate to normal and uremic rats.

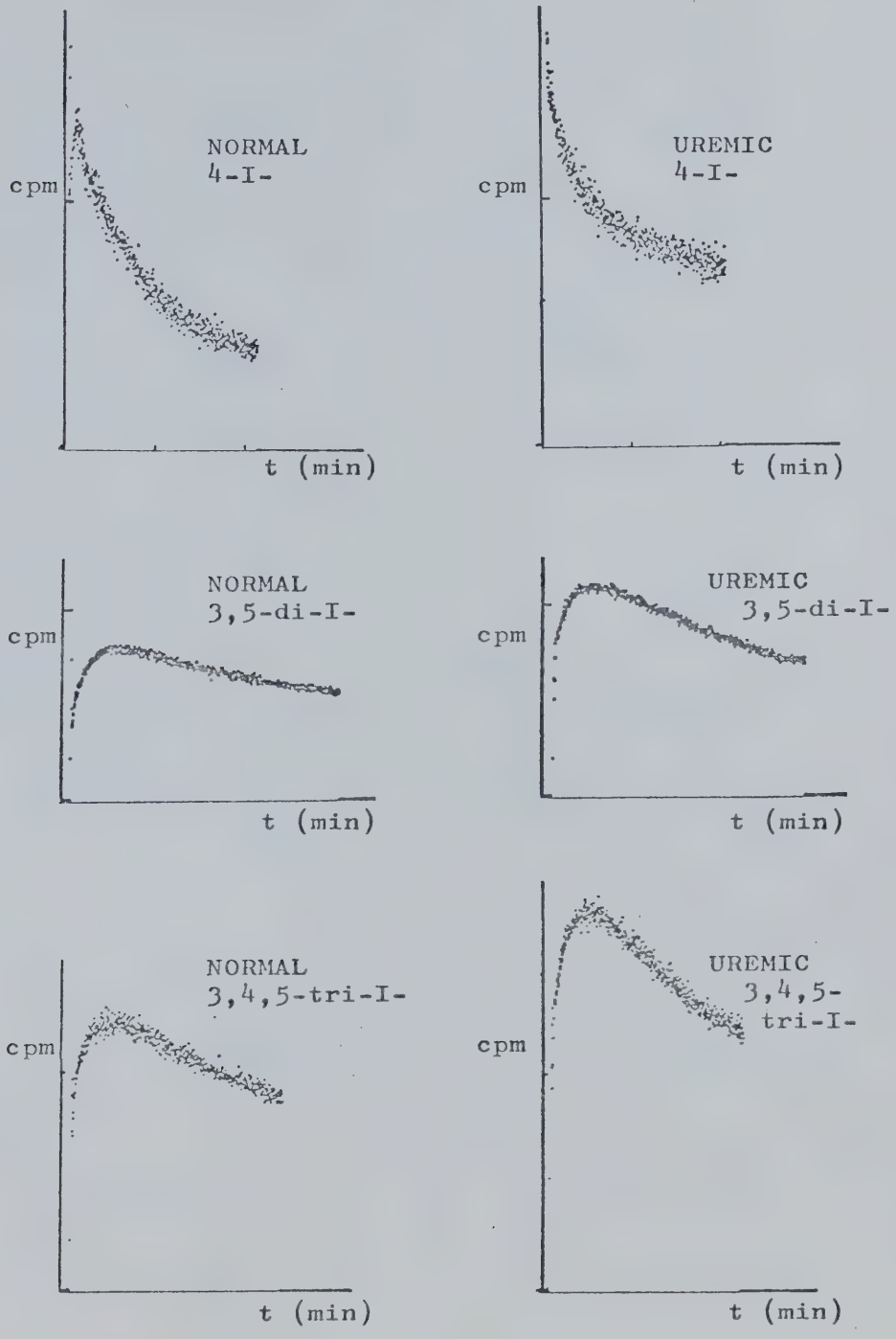


Figure9 . Typical renograms after i.v. adminstration of radioiodinated 4-iodo-, 3,5-diiodo-, and 3,4,5-tri-iodohippurates in normal and uremic rats.

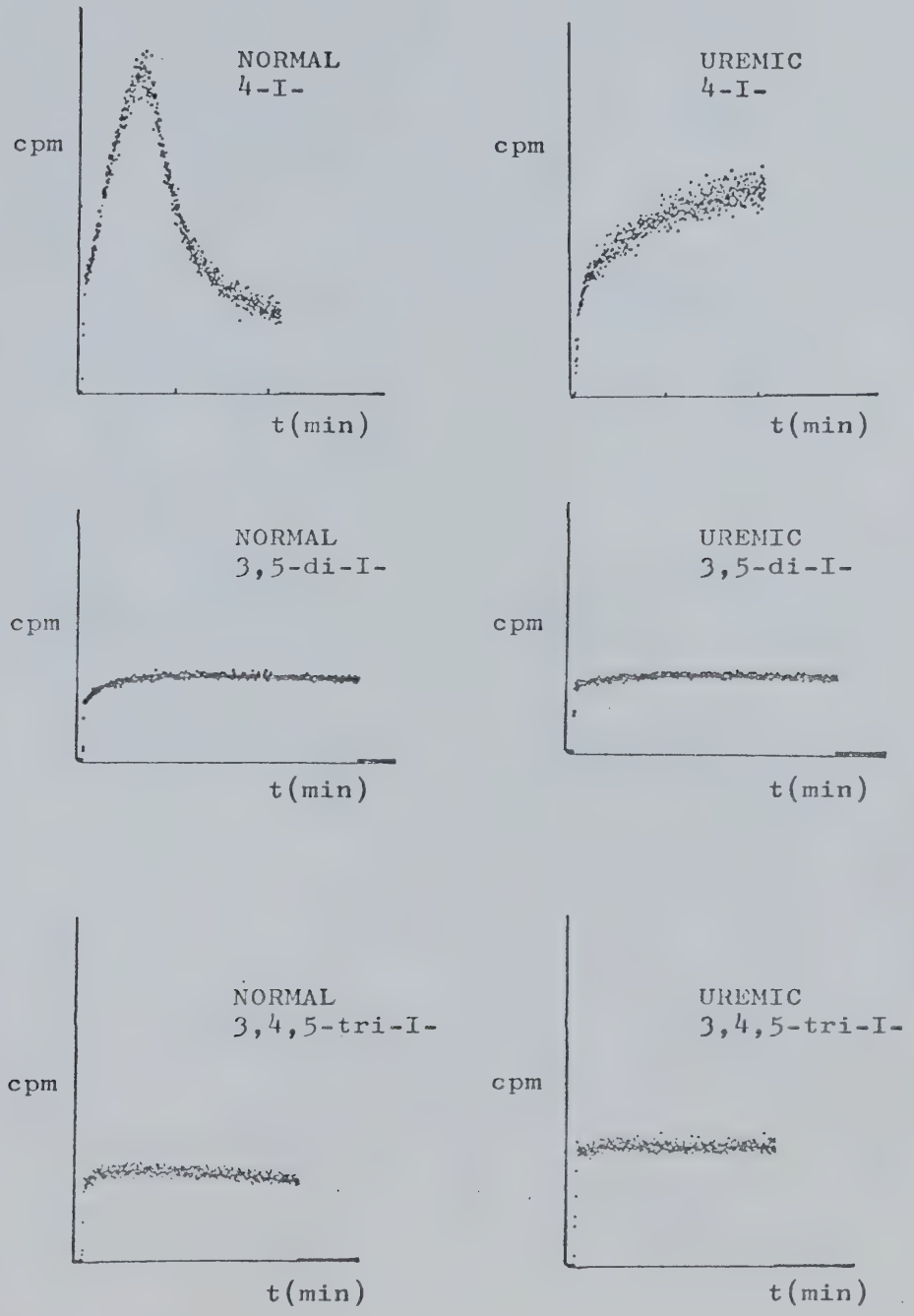


Figure10. Typical hepatograms after i.v. administration of radioiodinated 4-iodo-, 3,5 -diiodo-, and 3,4,5-triiodohippurate to normal and uremic rats.

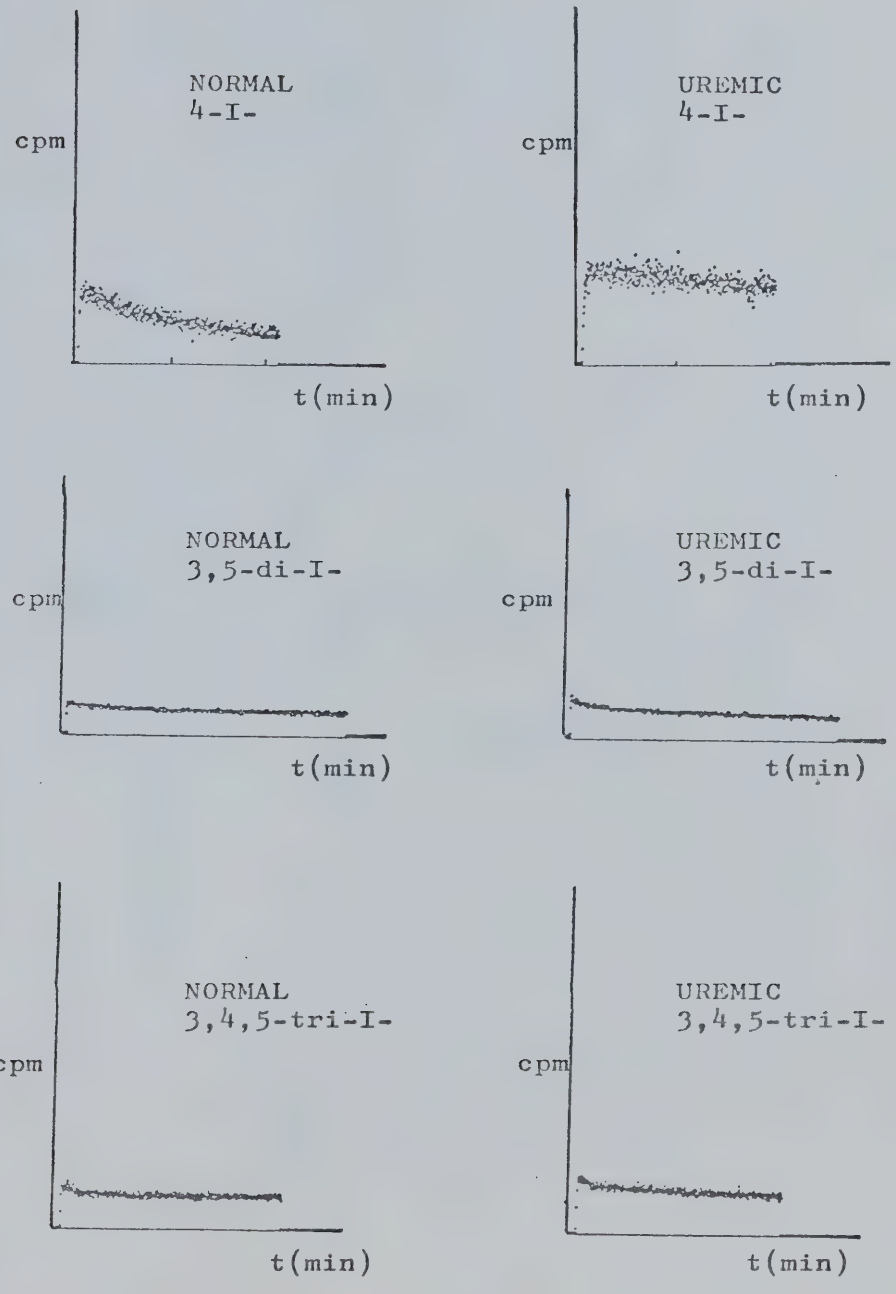


Table IV

Relative Liver and Kidney Weights and Serum Ca^{++} levels for Normal and uremic Rats[a]

Test Substance Group	Organ Weight as % of Whole Body				Serum Ca^{++} (mg%)	
	Normal Liver	uremic Liver	Normal Kidney	uremic Kidney	Normal	uremic
^{125}I-4-iodohippurate	3.3 ± 0.5	3.6 ± 0.5	0.5 ± 0.1	0.5 ± 0.1	8.7 ± o.4	7.5 ± 0.4
^{125}I-3,5-diiodohippurate	2.9 ± 0.5	3.7 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	8.3 ± 0.2	7.8 ± 0.2
^{125}I-3,45-triiodohippurate	2.9 ± 0.2	3.3 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	8.8 ± 0.8	7.8 ± 0.3
Mean Values	3.0 ± 0.4	3.5 ± 0.4*	0.5 ± 0.1	0.4 ± 0.1*	8.6 ± 0.5	7.7 ± 0.3*

(a) Data indicates mean values ± S.D. of 5 rats in each group.

* Significant difference between uremic and normal groups ($P \leq 0.01$)

Table V

Radioactivity Levels [a] in the Serum, [b] Whole blood, [c] liver, [d] total renal mass [e] and bile [f]

Radioactivity Measurement		Test Substance Group: ^{125}I-4-iodohippurate	^{125}I-3,5-diiodohippurate	^{125}I-3,4,5-triiodohippurate
Serum:				
	Normal	1.58 ± 0.3	3.22 ± 0.9	10.12 ± 1.6
	uremic	4.06 ± 0.5***	3.67 ± 0.7	8.58 ± 1.8
Wholeblood:				
	Normal	2.45 ± 0.3	5.70 ± 1.1	14.36 ± 1.9
	uremic	5.84 ± 1.0***	6.80 ± 0.9	14.31 ± 2.3
bile:	Normal	0.98 ± 0.3	8.98 ± 1.1	13.67 ± 5.9
	uremic	1.97 ± 0.5***	10.59 ± 3.4	19.67 ± 2.2
Liver:	Normal	2.78 ± 1.3	4.95 ± 1.1	5.75 ± 1.4
	uremic	3.31 ± 0.7***	6.22 ± 0.6**	7.52 ± 0.9**
Total renal mass:				
	Normal	9.54 ± 2.6	9.74 ± 1.7	10.62 ± 2.2
	uremic	10.45 ± 6.7	3.64 ± 1.8***	2.10 ± 0.5***

(a) expressed as percent of injected dose 25 minutes after i.v. administration data represents mean values ± S.D. of 5 rats in each group.

(b) Serum from 5 ml whole blood

(c) 5 ml whole blood

(d) entire liver (e) entire renal mass

(f) total bile collected during the 25 minute test period.

*** $p \leq 0.01$ ** $p \leq 0.025$

Table VI

Uptake and Elimination Data of ^{125}I-Labelled iodohippurates[(a)]

Parameter	Test Substance Group		
	^{125}I-4-iodohippurate	^{125}I-3,5-dioodohippurate	^{125}I-3,4,5-tri-iodohippurate
Liver Uptake Tmax (min)			
Normal	0.13 ± 0.03	2.90 ± 0.50	3.82 ± 0.45
uremic	0.11 ± 0.03	2.75 ± 0.34	4.64 ± 0.52*
Renal Uptake Tmax (min)			
Normal	6.40 ± 1.79	6.69 ± 1.44	14.47 ± 7.08
Uremic	17.40 ± 8.20**	14.27 ± 2.38***	22.95 ± 2.7*
Liver Excretion Rate Constant ($sec^{-1} \times 10^3$)			
Normal	1.066 ± 0.204	0.310± 0.050	0.325± 0.136
uremic	0.698 ± 0.219*	0.410± 0.042***	0.336± 0.037
Blood Clearance Rate Constant ($sec^{-1} \times 10^3$)			
Normal	0.696 ± 0.194	0.308± 0.109	0.580± 0.143
uremic	0.226 ± 0.09***	0.474± 0.190	0.340± 0.107**

(a) data derived from hepatograms, renograms and blood pool monitor following i.v. injection of ^{125}I-iodohippurates. Each value represents the mean ± S.D. from 5 rats in each test group.

* * $p \leq 0.05$
** $p \leq 0.025$
*** $p \leq 0.01$

Discussion of Results

It is well documented that mono-iodohippuric acid is excreted via the kidneys in normal body function. The appearance, concentration and excretion of the tracer in the kidney was detected, recorded and evaluated during the experiment. The characteristic curve recorded as a renogram displayed the general function of the kidney, and the renal glomerular filtration and renal tubular cell secretion were primarily involved in the elimination of the mono-iodohippuric acid, or hippurate (103).

A large component of the steep rise in the first renogram segment represented the mixing and blood background activity. The slowly rising phase followed, represented the build-up in radioactivity in the kidney-pelvis complex related to the delivery of iodo-hippurate to intrarenal vessels, its active build-up in proximal tubule cells and its passage into urine in tubular lumina and subsequently the kidney pelvis. This segment ended at a point called the kidney uptake maximum and the renogram curve started to fall when and only when the rate at which radioactivity, leaving the kidney-pelvis complex, exceeded the rate at which radioactivity entered. Some of the important factors, like the state of renal blood vessels including glomerular capillaries, the active transport function of tubules, the intrarenal urine flow rate, and the emptying of the

calyceal-pelvic system would influence the segment, especially the point stated as kidney uptake maximum. The rate of descent in the renogram represented the rate of decline of total iodo-hippurate in the kidney-pelvis complex (100). The doses of tracer employed compared to the dose needed to approach the tubular transport maximum was small, therefore, except in severe renal failure, tubular transport was not a limiting factor (100). The blood excretion constant expressed the rate of decline of iodohippurate concentration in plasma and was approximately exponential during the period under study (100,120)

The renograms obtained from normal rats after single dose with I-125-4-iodohippurate showed one difference from human renograms, in that only two out of the five rats tested showed a descending segment in their renograms within the 25 minute period of observation. However, this would not be inconsistant with the known very slow formation of urine in the rat. The uptake phase of the renogram was present, and it was therefore possible to calculate the uptake maximum values which could then be correlated to blood clearance rate constants.

The kidney uptake maximum observed in the normal rats averaged at about 6.39 minutes and the rate of mono-iodohippurate eliminated from the blood expressed as the blood excretion constant in the same animals recorded with a

mean of 6.96×10^{-4} . These data agreed with the observation by Nordyke (1) in the experimental observation from normal individuals. The rapid liver uptake maximum at about 0.127 minutes in normal rats with a fast average of liver excretion constant at 1.066×10^{-3} indicated primarily the perfusion rate of blood flow into and out of the hepatic system. Only 0.98 % of the total activity was found in the bile collected over the whole scanning period, indicating the minimum involvement of the liver in the elimination of mono-iodohippurate in normal animals.

However, in the animals with renal failure, the kidney uptake maximum was delayed to over 17.44 minutes (about 3 times the normal), while the blood excretion constant had an average of 2.26×10^{-4} (about 1/3 of the normal). Bearing in mind that the animal had only one-third of the left kidney left in the body by the surgical induction of renal failure, we kept on studying other parameters. Although the average liver uptake maximum (0.113 minutes) was similar to the normal animals, the mean liver excretion constant was about 6.98×10^{-4}, slower than the normal. Total activity found in the bile during the scanning period increased to an average at 1.97 % of the total activity. The average activity of the excised liver after scanning was 3.3%, compare to normal 2.7%, indicating a slight increase of liver involvement in the excretion of mono-iodohippurate in animals with renal failure. This may have been due to

certain changes of excretion route or some unknown compensating mechanisms in the condition of renal failure. Diminished kidney function in the surgically induced renal failure model was illustrated by a delayed kidney uptake maximum and a high residual activity found in the kidney (average at 10.45% compared to 4.77% in normals) after the scanning period. The delayed excretion and accumulation of iodohippurate in the kidney with chronic renal failure was not surprising. Different reports had already expressed the same observation (101, 102, 103). Rosenthall (107) found that a high concentration of Hippuran® remained fixed in the renal parenchyma in some cases of renal failure. Gault (100) suggested that tubular transport insufficiency in renal failure might be one of the major factor. It is interesting to note that even the consequence of renal insufficiency and uremia had also considerable effect on the kidney itself. White (121) reported that uremic rats serum depressed the uptake of para-aminohippurate (similar biological property as iodohippurate) by normal rat kidney cortex slices, while normal rat serum increased the uptake of para-aminohippurate.

Finally, the radioactivity remaining in the whole blood was 5.84% (compared to 2.45%), expressing the overall increased difficulty in the animals with renal failure to eliminate the mono-iodohippurate in the same period of time as normal animals.

Upon increased iodination, the kidney uptake phase, as compared with that for mono-iodohippurate, was progressively extended in duration, a trend observed in both normal and uremic rats. However, the effect was much more pronounced in normal than in uremics. The mono to di and mono to tri iodohippurate kidney uptake time maximum values in normals represented respective increases of 4.8% and 126% and in uremics represented a decrease of 18% and an increase of 31.6% respectively. It can be concluded from this data that the effect of increasing molecular weight on renal uptake is more pronounced in normal rats than in chronic renal failure. Alternately, it may be stated that the effects of decreased functional renal mass on renal uptake were greatly magnified at low M.W. (273% increase) and has increased importance for high M.W. compounds (159% increase); the effect for the di-iodohippurate was intermediate (213% increase). The elimination phase of the renograms obtained after dosing with di- or tri-iodohippurate also did not display a negative slope. In order to study the elimination phase, diuresis would have to be stimulated, perhaps simply by loading with saline.

The hepatograms obtained all displayed uptake and elimination phases. They were simplified by the bile duct cannulation procedure which allowed the removal of bile, thereby avoiding the complex effects resulting from

deposition of radioactive bile into the intestine in the field of the NaI scintillation detector placed immediately over the liver. The hepatograms for the di- and tri-iodohippurates and the data derived from the hepatograms were compatible with observed biliary elimination. Although no attempt was made to isolate and quantitate the influence of hepatic perfusion or uptake phase, it was apparent from the data that biliary elimination became increasingly important as the molecular weight increased from 326 through 451 to 571. This observation agreed with the investigation made previously by Williams (4), for other structurally related compounds. It was also shown that the role of the liver was increased significantly in animals with chronic renal failure.

Residual radioactivity (after 25 minutes) in blood, liver and renal mass was also in agreement with these interpretations of both renograms and hepatograms, and with the biliary excretion data. Residual liver radioactivity thus increased from mono- to di- to tri- iodohippuric acid in both uremic and normal rats. Blood radioactivity levels from mono-iodohippurate in normals were approximately 50% of that in uremics, whereas for tri-iodohippurate they were nearly equal, suggesting again that the liver became more involved as molecular weight increased and that the uremic liver was compensating better for high molecular weight compounds than the normal liver.

Radioactivity remaining in the normal kidney did not support data derived from the renograms in that there was no change with increasing molecular weight whereas the renal uptake maximum did show a molecular weight effect. Radioactivity remaining in the residual (uremic) renal mass was drastically reduced as the molecular weight increased. This observation, and the observation of increased renal uptake maximum would be compatible with decreased renal function (uptake) upon increase in molecular weight over the range studied.

It has been known that one of the major routes of excretion of foreign compounds in the body is by way of the kidney, which implies the presence or the formation of a water-soluble substance. Following glomerular filtration, tubular re-absorption into plasma is virtually complete for substances with a high partition coefficient (lipid:water). Since most active drugs (by virtue of their ability to penetrate cellular membranes) are lipid soluble, metabolic conversion, usually in the liver, to a more polar form is essential for their excretion. Presumably, a lipid membrane surrounds the liver microsomes in which are found the non-specific enzyme systems responsible for most metabolic conversions. The membrane is readily penetrated by the lipophilic drug, and metabolism to a more polar form results, followed by increased excretion during the next

passage through the kidney generally (122). This solubility, and partition coefficients, play a role of primary importance in determining the excretion and metabolism of a foreign compound.

In their recent study on the factors influencing renal excretion of a o-iodohippurate, Bryan and Mahr suggested the existance of a passive re-absorption of o-iodohippurate in the renal tubules on the basis of nonionic diffusion (123) although the rate is much smaller than the active secretion and only net excretion was observed in small amounts of tracer being used. Their findings, indicated that iodohippurate like several other typical organic acids, are believed to undergo bi-directional transport by a combination of active secretion and passive re-absorption (123,124).

The physical properties of the three different iodohippurates provide further support of the possibilities that non-specific tubular re-absorption causes the change of excretion route. Ortho-iodohippurate has a dissociation constant (pKa) 3.63 and partition coefficient (Kp chloroform:water) 0.222. However, addition of one or more halogen atomic iodine to the o-iodohippurate increases the partition coefficient of the molecule as well as its total molecular weight. This increase of lipid solubility especially observed in the tri-iodohippurate might result in

a possible great increase in renal tubular re-absorption, and therefore causes an increase in liver uptake.

The role of the liver in elimination of the xenobiotics 4-iodohippurate (I-125), 3,5-diiodohippurate (I-125) and 3,4,5-triiodohippurate (I-125) increased as the molecular weight of the substrate increased. This effect was more pronounced in subtotally nephrectomized chronically uremic rats than in normal rats. Conversely, renal elimination became quantitatively less important as the molecular weight increased, an effect more pronounced in the uremic rats than in the normal rats. The net result was that the amount of high molecular weight tracer radioactivity in the bile was 69% greater in uremics than in normal rats. This effect, if present in clinical chronic renal failure, could be particularly important in a therapeutic regimen if the excreted material is extensively recirculated through the hepato-biliary system. In a more positive sense, it could be in an extremely valuable compensatory mechanism, preventing build-up of body levels of the xenobiotic which cannot be eliminated by the normal renal route.

V. Summary and Conclusions

1. A state of chronic renal failure was induced in rats by long term maintenance of animals which had undergone right sub-total nephrectomy followed by contralateral nephrectomy ten days later.

2. The model of chronic renal failure was characterized by altered hepatic weight, altered renal mass and decreased serum calcium levels in addition to the qualitative changes observed in the rat following sub-total and contralateral nephrectomy.

3. I-125 labeled rose bengal, Tc-99m labeled sulfur colloid, I-125 labeled 4-iodohippurate and its higher molecular weight analogues 3,5-diiodohippurate and 3,4,5-triiodohippurate, were employed in different excretion studies in both uremic and normal control rats.

4. Clearance of I-125 rose bengal from the blood, following intravenous injection, was determined to proceed at a significantly slower rate in uremic rats than in normal controls.

5. Total biliary elimination of I-125 rose bengal was also reduced in uremic rats than in normals.

6. Significant linear correlation was found between serum calcium levels and I-125 rose bengal clearance rate constants during the one hour elimination.

7. A significant increase in Tc-99m sulfur colloid

clearance from the blood and significant increase in hepatic uptake rate constant were observed in uremic rats over normal controls.

8. The hepatic elimination of the radioactive I-125 labeled 4-iodohippurate, 3,5-diiodohippurate and 3,4,5-triiodohippurate, was observed to be increased as the molecular weight of the substrate increased. This effect was more pronounced in subtotally nephrectomized chronically uremic rats than in normal rats.

9. Renal elimination of the I-125 iodohippurate analogues became quantitatively less important as the molecular weight increased, an effect more pronounced in the uremic rats than in the normal rats.

10. These findings might be of significance in the understanding of clinical chronic renal failure, elimination of xenobiotics in impaired renal state and the possible application in the adjustment of therapeutic regimen in the treatment of chronic renal failure.

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